

USSN 10/663,853

Docket No. 3226-A

What is claimed is:

1. (Previously amended) A method for producing a protein comprising culturing Chinese hamster ovary (CHO) cells that produce the protein in the presence of a cytidine analogue in the culture medium, wherein the presence of the cytidine analogue in the culture medium results in an increase in the production of the protein and wherein the culture medium is serum free.
2. (Original) The method of claim 1, wherein the CHO cells are cultured at a temperature from about 29°C to 36°C.
3. (Currently amended) The method of claim 1, wherein the cytidine analogue is 5-aza-2'-deoxycytidine with or without one or more phosphate group(s) attached to the 3' and/or 5'_carbon(s).
4. (Currently amended) The method of claim 3, wherein the concentration of 5-aza-2'-deoxycytidine with or without one or more phosphate group(s) attached to the 3' and/or 5'_carbon(s) is from about 0.1 μ M to about 50 μ M.
5. (Currently amended) The method of claim 1, wherein the cytidine analogue is 5-bromo-2'-deoxycytidine with or without one or more phosphate group(s) attached to the 3' and/or 5'_carbon(s).
6. (Currently amended) The method of claim 5, wherein the concentration of 5-bromo-2'-deoxycytidine with or without one or more phosphate group(s) attached to the 3' and/or 5'_carbon(s) is from about 0.1 μ M to about 50 μ M.
7. (Original) The method of claim 1, wherein the cytidine analogue in the medium is at a concentration from about 0.01 μ M to about 100 μ M.
8. (Original) The method of claim 1, wherein the protein is an antibody.
9. (Original) The method of claim 1, wherein the protein is a recombinant protein.

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10. (Original) The method of claim 1, wherein the messenger RNA encoding the protein is transcribed starting from a viral promoter.

11. (Original) The method of claim 1, wherein the volume of the cell culture is at least about 100 liters.

12. (Original) The method of claim 1, wherein the protein is secreted into the medium.

13. (Canceled)

14. (Previously amended) A method for producing a recombinant protein comprising

culturing mammalian cells in a medium comprising a DNA demethylating agent at a temperature from about 29°C to 36°C, wherein the recombinant protein is secreted into the medium by the mammalian cells, and

harvesting the medium comprising the recombinant protein,
wherein the mammalian cells have been genetically engineered to produce the recombinant protein, and

wherein the presence of the DNA demethylating agent in the medium results in an increase in the amount of the recombinant protein in the medium.

15. (Original) The method of claim 14, wherein the DNA demethylating agent is a cytidine analogue.

16. (Currently amended) The method of claim 15, wherein the cytidine analogue is 5-aza-2'-deoxycytidine with or without one or more phosphate group(s) attached to the 3' and/or 5' carbon(s).

17. (Currently amended) The method of claim 16, wherein the concentration of 5-aza-2'-deoxycytidine with or without one or more phosphate group(s) attached to the 3' and/or 5' carbon(s) is from about 0.1 μM to about 50 μM.

18. (Original) The method of claim 15, wherein the cytidine analogue is at a concentration from about 0.01 μM to about 100 μM.

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19. (Currently amended) The method of claim 15, wherein the cytidine analogue is 5-bromo-2'-deoxycytidine with or without one or more phosphate group(s) attached to the 3' and/or 5'_carbon(s) at a concentration from about 0.1 μ M to about 50 μ M.

20. (Canceled)

21. (Original) The method of claim 14, wherein the medium is serum free.

22. (Original) The method of claim 14, wherein the mammalian cells are CHO cells.

23. (Original) The method of claim 14, wherein the recombinant protein is an antibody.

24. (Original) A method for producing a recombinant protein comprising culturing CHO cells in a medium comprising 5-aza-2'-deoxycytidine at a concentration from about 1.0 μ M to about 20 μ M at a temperature of less than 37°C, wherein the CHO cells secrete the recombinant protein into the medium and wherein the medium is serum free, and collecting the medium comprising the recombinant protein.

25. (Original) A method for producing a recombinant protein comprising culturing CHO cells in a medium comprising 5-bromo-2'-deoxycytidine 5'-monophosphate at a concentration from about 1.0 μ M to about 20 μ M at a temperature of less than 37°C, wherein the CHO cells secrete the recombinant protein into the medium and wherein the medium is serum free, and collecting the medium comprising the recombinant protein.

26. (Currently amended) A CHO cell culture comprising:
a medium comprising 5-aza-2'-deoxycytidine with or without one or more phosphate group(s) attached to the 3' and/or 5'_carbon(s) at a concentration from about 0.1 μ M to about 50 μ M, wherein the medium is serum free; and
a CHO cell line that has been genetically engineered to express a recombinant, secreted protein.

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27. (Currently amended) A mammalian cell culture comprising:
a medium comprising 5-aza-2'-deoxycytidine with or without one or more phosphate group(s) attached to the 3' and/or 5'_carbon(s) at a concentration from about 0.1 μ M to about 50 μ M, wherein the medium is serum free; and
a mammalian cell line that can express an antibody.
28. (Currently amended) A CHO cell culture comprising:
a medium comprising 5-bromo-2'-deoxycytidine with or without one or more phosphate group(s) attached to the 3' and/or 5'_carbon(s) at a concentration from about 0.1 μ M to about 50 μ M, wherein the medium is serum free; and
a CHO cell line that has been genetically engineered to express a recombinant, secreted protein..
29. (Currently amended) A mammalian cell culture comprising:
a medium comprising 5-bromo-2'-deoxycytidine with or without one or more phosphate group(s) attached to the 3' and/or 5'_carbon(s) at a concentration from about 0.1 μ M to about 50 μ M, wherein the medium is serum free; and
a mammalian cell line that can express an antibody.
30. (Currently amended) A method for producing a protein comprising culturing CHO cells that produce the protein in the presence of 5-aza-2'-deoxycytidine with or without one or more phosphate group(s) attached to the 3' and/or 5'_carbon(s) at a temperature from about 29°C to 36°C, wherein the presence of the 5-aza-2'-deoxycytidine results in an increase in the production of the protein.
31. (Currently amended) A method for producing a protein comprising culturing CHO cells that produce the protein in the presence of 5-bromo-2'-deoxycytidine with or without one or more phosphate group(s) attached to the 3' and/or 5'_carbon(s), wherein the presence of the 5-bromo-2'-deoxycytidine results in an increase in the production of the protein.